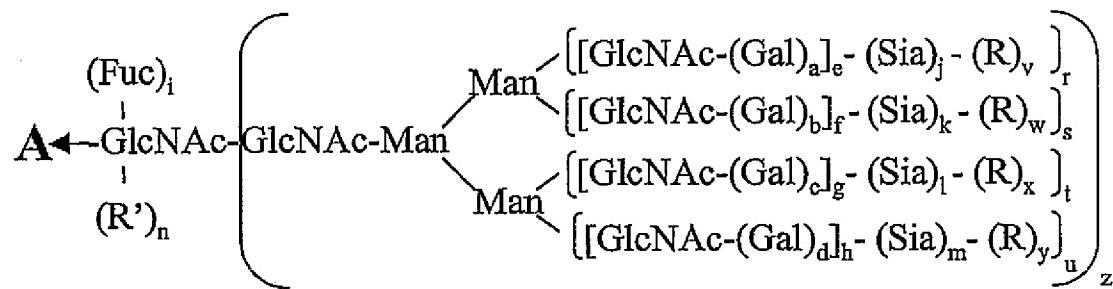
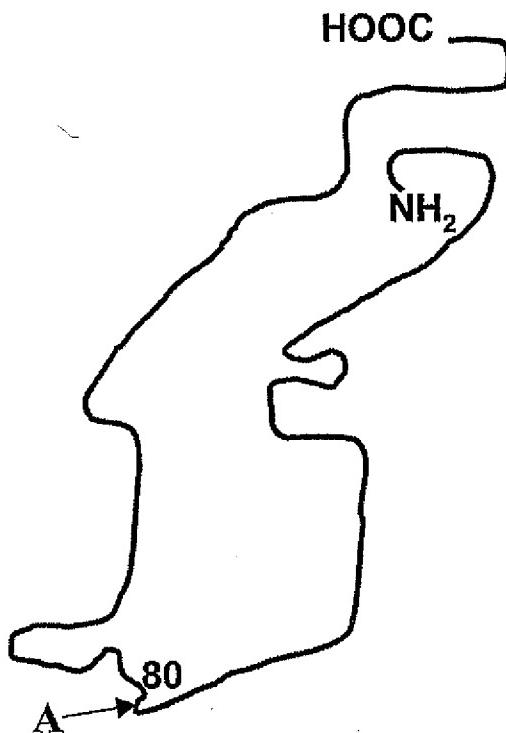


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a-d, i, r-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 or 1.

n, v-y = 0; z = 0 or 1; R = polymer

FIG. 29A

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CHO, BHK, 293 cells, Vero expressed IF-beta  
 $h = 1 \text{ to } 3;$   
 $a-g, j-m, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $n, v-y = 0; z = 1.$

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3

$h = 1 \text{ to } 3;$   
 $a-g, i$  (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 or 1;  
 $j-m, v-y$  (independently selected) = 0 or 1;  
 $z = 1; n = 0; R = PEG.$

## FIG. 29B

Insect cell expressed IF-beta  
 $a-d, f, h, j-n, s, u, v-y = 0;$   
 $e, g, i, r, t$  (independently selected) = 0 or 1;  
 $z = 1.$

- ↓  
 1. GNT's 1&2, UDP-GlcNAc  
 2. Galactosyltransferase, UDP-Gal  
 2. CMP-SA-PEG, ST3Gal3,  
 buffer, salt

$b, d, f, h, k, m, n, s, u, w, y = 0;$   
 $a, c, e, g, i, r, t$  (independently selected) = 0 or 1;  
 $j, l, v, x$  (independently selected) = 0 or 1;  
 $z = 1; R = PEG.$

## FIG. 29C

86/345

Yeast expressed IF-beta  
a-n = 0; z = 1;  
r-y (independently selected) = 0 to 1;  
R (branched or linear) = Man, oligomannose or  
polysaccharide.

- ↓  
1. Endo-H  
2. Galactosyltransferase, UDP-Gal  
3.. CMP-SA-PEG, ST3Gal3

a-m, r-z= 0; n = 1; R' = -Gal-Sia-PEG.

## FIG. 29D

CHO, BHK, 293 cells, Vero expressed IF-beta  
h = 1 to 3;  
a-g, j-m, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
n, v-y = 0; z = 1.

- ↓  
1. CMP-SA-PEG, ST3Gal3

h = 1 to 3;  
a-g, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
j-m, v-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

## FIG. 29E

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Insect cell expressed IF-beta  
a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t  
(independently selected) = 0 or 1; z = 1.

- ↓
1. GNT's 1,2,4,5, UDP-GlcNAc
  2. Galactosyltransferase, UDP-Gal
  3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

## FIG. 29F

Yeast expressed IF-beta  
a-n = 0; z = 1;  
r-y (independently selected) = 0 to 1;  
R (branched or linear) = Man, oligomannose.

- ↓
1. mannosidases
  2. GNT's 1,2,4,5, UDP-GlcNAc
  3. Galactosyltransferase, UDP-Gal
  - 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

## FIG. 29G

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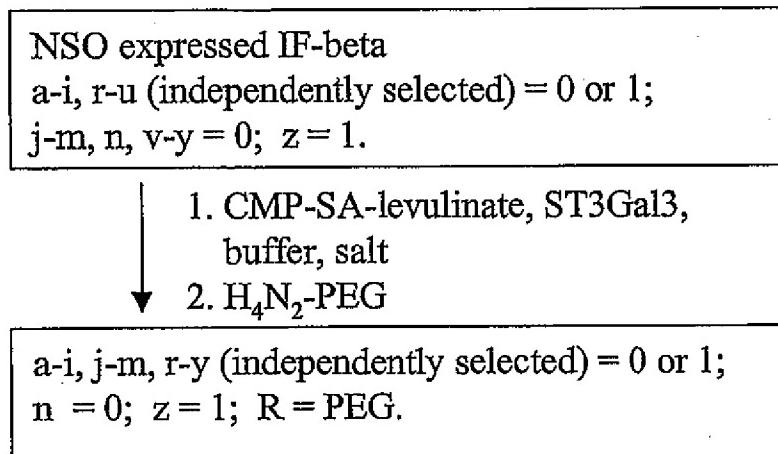


FIG. 29H

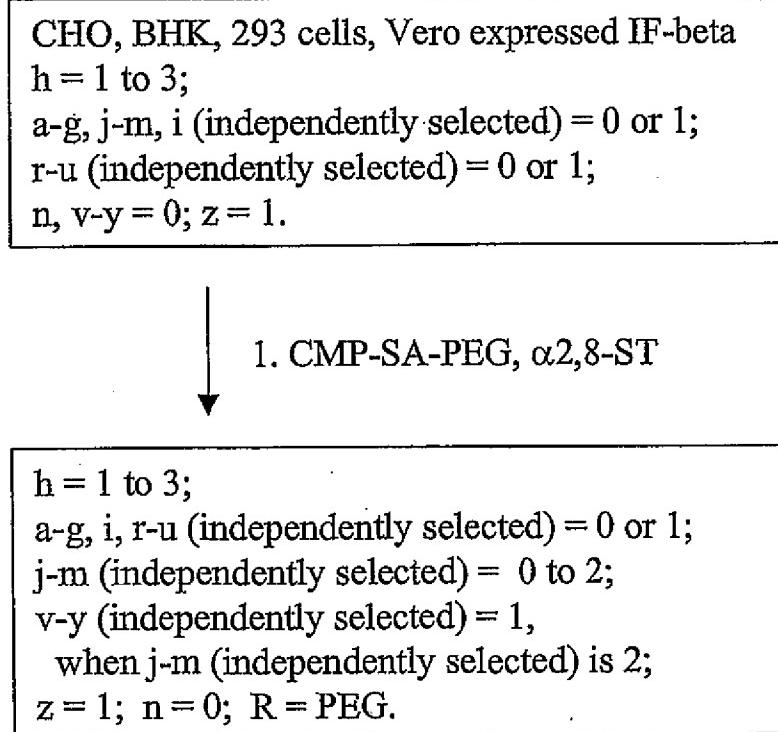


FIG. 29I

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CHO, BHK, 293 cells, Vero expressed IF-beta  
a-g, j-m, r-u (independently selected) = 0 or 1;  
h = 1 to 3; n, v-y = 0; z = 1.



1. Sialidase
2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1;  
h = 1 to 3; n = 0; z = 1; R = PEG.

## FIG. 29J

CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.



1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n=0;  
v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 29K

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NSO expressed Ifn-beta.  
 a-d, i-m, r-u, z (independently selected) = 0 or 1;  
 e-h = 1; n, v-y = 0;  
 Sia (independently selected) = Sia or Gal.

- 1. Sialidase and  $\alpha$ -galactosidase
- 2.  $\alpha$ -Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
 e-h = 1; R = PEG  
 n = 0; v-y (independently selected) = 1,  
 when j-m (independently selected) is 1;

FIG. 29L

CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
 a-d, i-m, r-u, z (independently selected) = 0 or 1;  
 e-h = 1; n, v-y = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq),  
 ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
 e-h = 1; n = 0;  
 v-y (independently selected) = 0 or 1; R = PEG.

FIG. 29M

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CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓  
1. CMP-SA-levulinic acid, ST3Gal3,  
buffer, salt  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n = 0;  
v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 29N

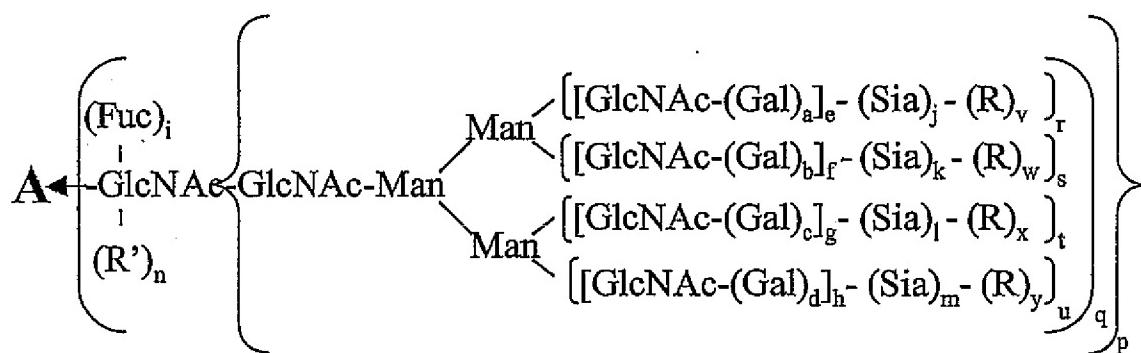
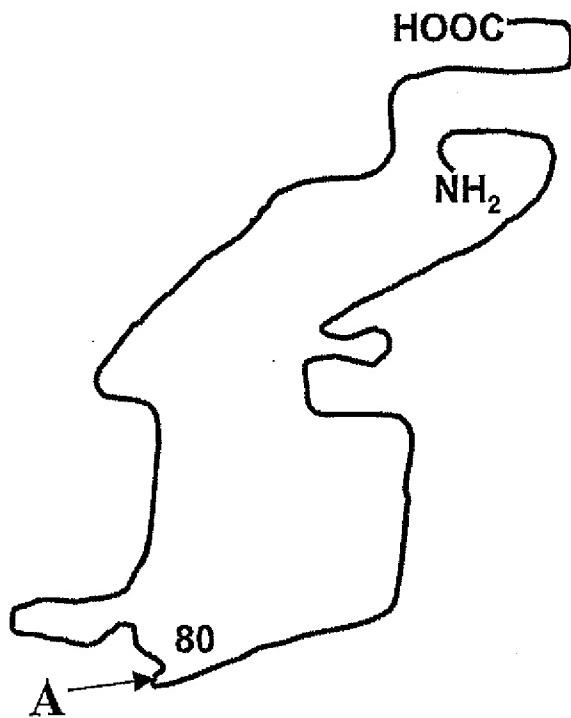
CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, r-u, z (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓  
1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
n, v-y (independently selected) = 0.

## FIG. 29O

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a-d, i, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

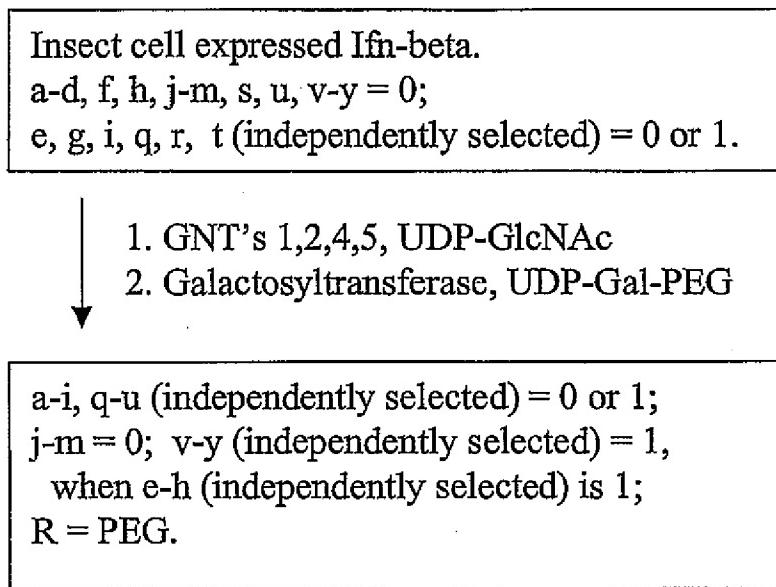
j-m (independently selected) = 0 to 100.

v-y = 0; R = modifying group;

R' = H, glycosyl group, modifying group, glycoconjugate.

FIG. 29P

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## FIG. 29Q

Yeast expressed Ifn-beta.  
a-m = 0; q-y (independently selected) = 0 to 1;  
p = 1;  
R (branched or linear) = Man, oligomannose.

↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0;  
n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

## FIG. 29R

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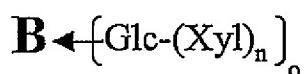
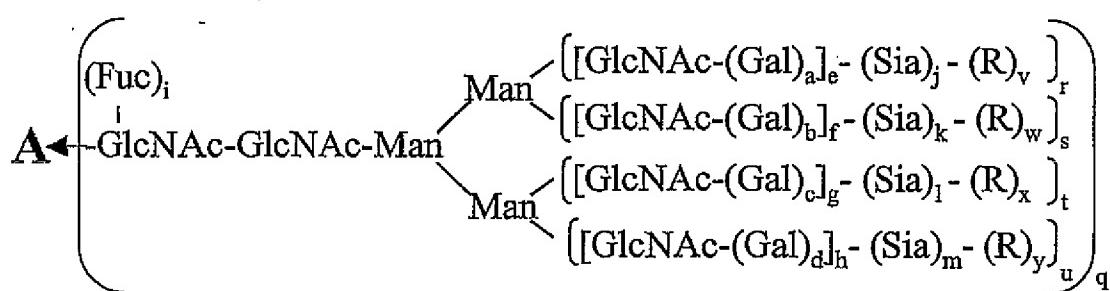
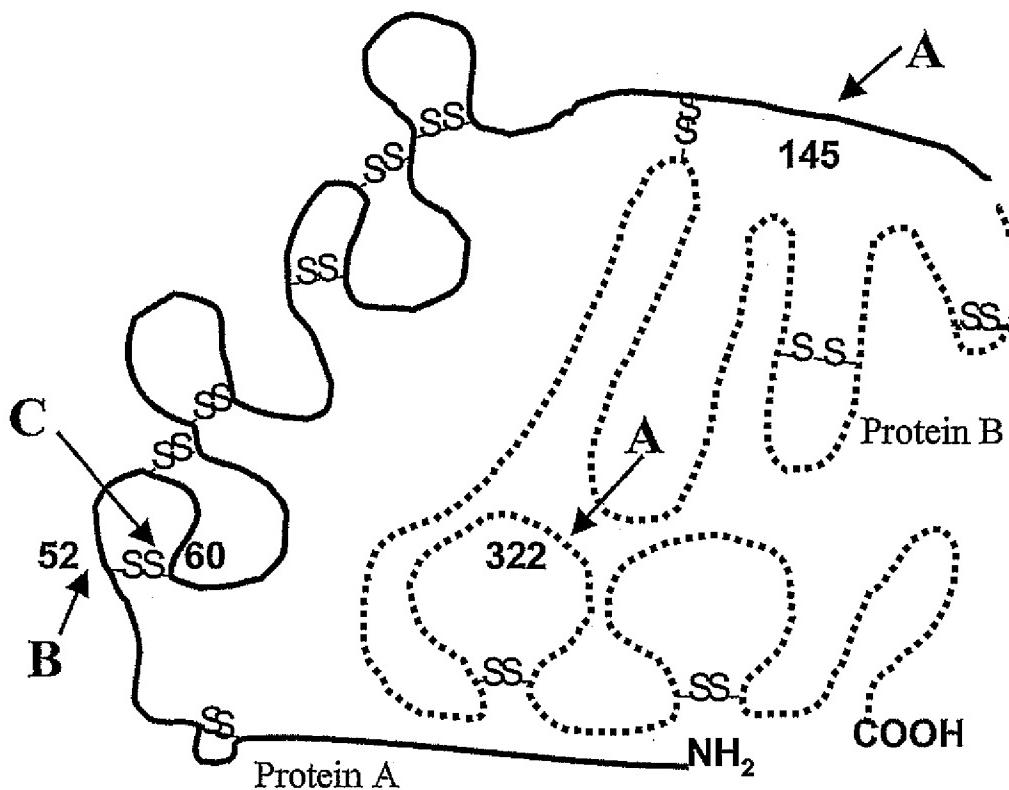
CHO, BHK, 293 cells, Vero expressed Ifn-beta.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP,  
ST3Gal3
  2. ST3Gal3, desialylated transferrin.
  3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 29S

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a-d, i, q-u (independently selected) = 0 or 1.  
o, p (independently selected) = 0 or 1.

e-h, n (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-y = 0;

R = modifying group, mannose, oligo-mannose, Sia-Lewis X, Sia-Lewis A..

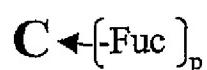


FIG. 30A

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BHK expressed Factor VII or VIIa  
 a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG (16 mole eq),  
 ST3Gal3

a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;  
 v, x, (independently selected) = 1,  
 when j, l (respectively, independently selected) is 1;  
 R = PEG.

## FIG. 30B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa  
 a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG (1.2 mole eq),  
 ST3Gal3  
 3. CMP-SA (8 mol eq), ST3Gal3

a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;  
 r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;  
 v or x, (independently selected) = 1,  
 when j or l, (respectively, independently selected) is 1;  
 R = PEG.

## FIG. 30C

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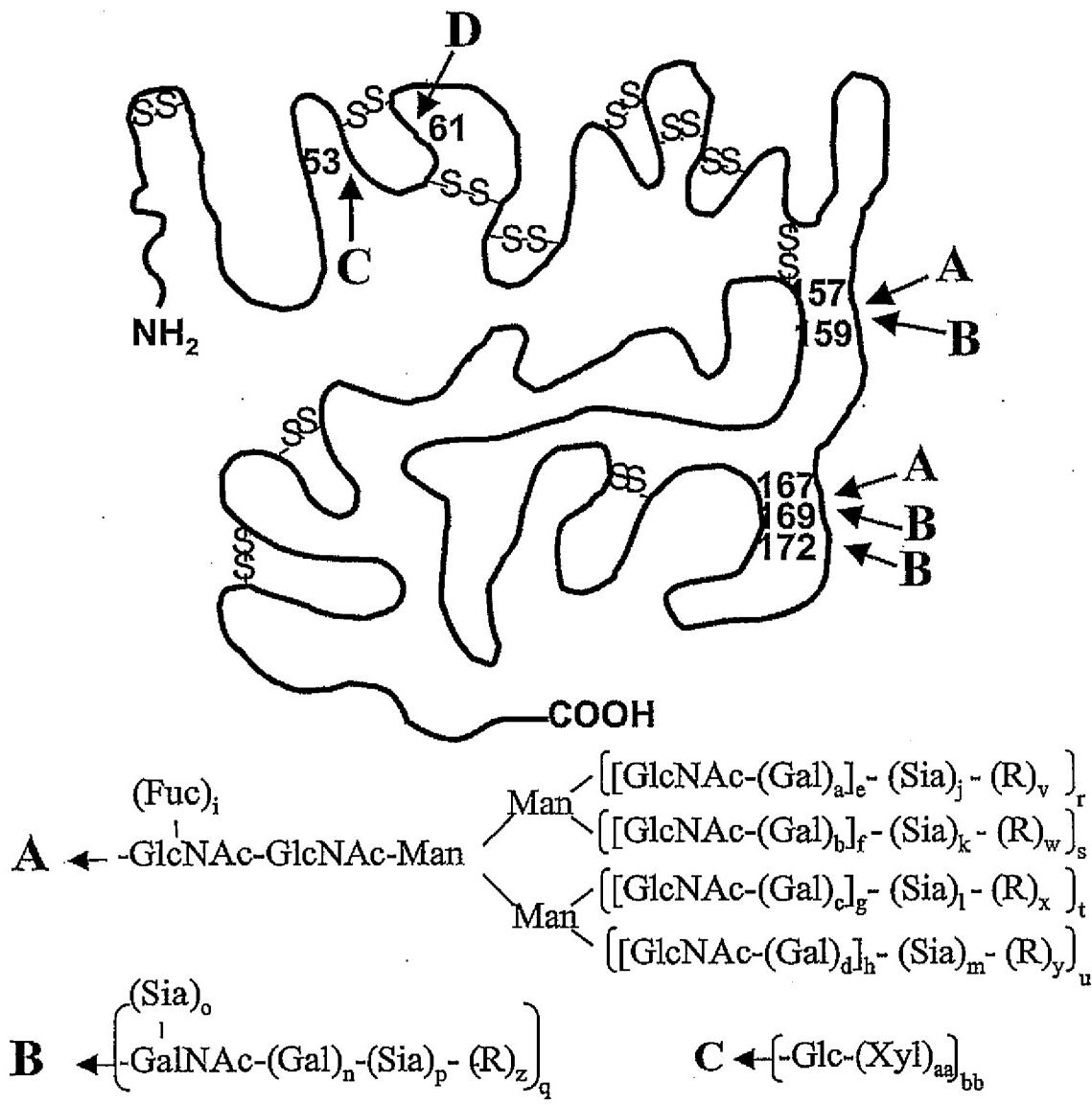
NSO expressed Factor VII or VIIa  
a--u (independently selected) = 0 or 1;  
v-y = 0; n = 0-4;  
Sia (independently selected) = Sia or Gal.

- ↓
1. Sialidase and  $\alpha$ -galactosidase
  2. Galactosyltransferase, UDP-Gal
  3. CMP-SA-PEG, ST3Gal3

a-m, o-u (independently selected) = 0 or 1;  
n = 0-4; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
Sia = Sia; R = PEG.

FIG. 30D

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a-d, i, n-u (independently selected) = 0 or 1.

bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.

e-h, aa (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 31A

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CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1;  
 R = PEG.

## FIG. 31B

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG, ST3Gal3  
 3. ST3Gal1, CMP-SA

a-d, n, p, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1;  
 o, z = 0; R = PEG.

## FIG. 31C

100/345

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i,  
 o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

a-d, n, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =  
 0 or 1; R = PEG;  
 o, v-y, gg = 0;  
 j-m, p, ee (independently selected) = 0 or 1, but when  
 p = 1, z = 1.

## FIG. 31D

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently  
 selected) = 0 or 1;  
 v-z, gg = 0.

CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)  
 = 0 or 1; R = PEG;  
 o, p, z = 0; j-m, ee, v-y, gg (independently selected) =  
 0 or 1.

## FIG. 31E

101/345

CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. CMP-SA-levulinate, ST3Gal3,  
 buffer, salt  
 2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0; R = PEG;  
 j-m, ee, v-y, gg (independently selected) = 0 or 1.

FIG. 31F

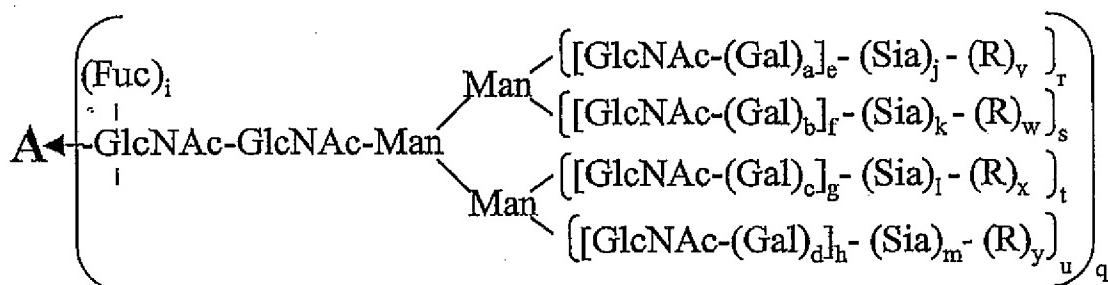
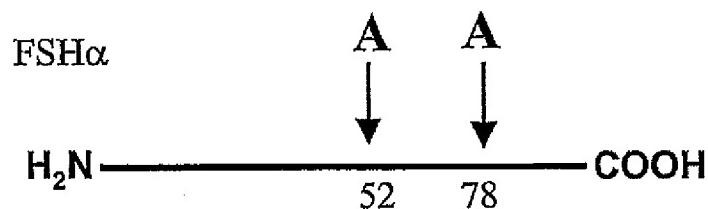
CHO, BHK, 293 cells, Vero expressed Factor IX  
 a-d, n, q, bb, cc, dd, ff = 1;  
 e-h, aa = 1 to 4;  
 ee, j-m, i, o, p, r-u (independently selected) = 0 or 1;  
 v-z, gg = 0.

- ↓  
 1. CMP-SA-PEG, α2,8-ST

a-d, q = 1; e-h = 1 to 4;  
 aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1;  
 o, p, z = 0; R= PEG;  
 j-m, ee (independently selected) = 0 to 2;  
 v-y, gg (independently selected) = 1, when j-m (independently selected) is 2;

FIG. 31G

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a-d, i, q-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose.

FIG. 32A

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CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 32B

CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 32C

104/345

NSO expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0;  
Sia (independently selected) = Sia or Gal.

- ↓  
1. Sialidase and  $\alpha$ -galactosidase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal1

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 32D

CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3  
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 32E

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CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt  
↓ 2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 32F

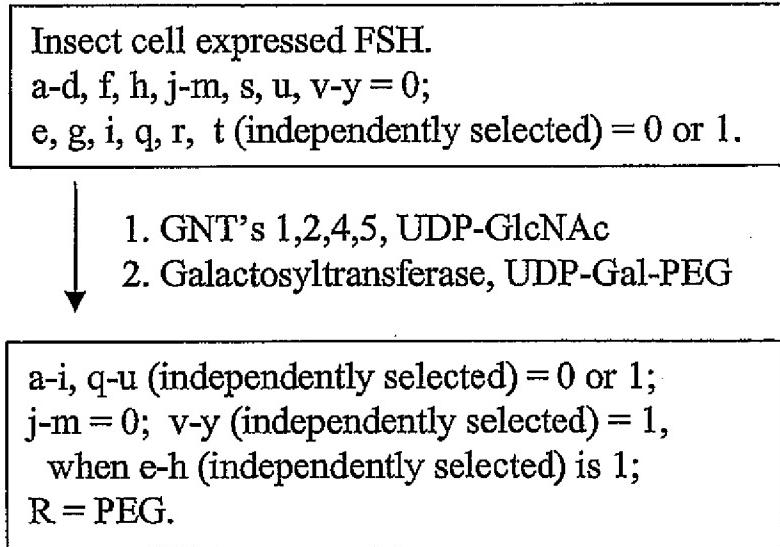
CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA, α2,8-ST

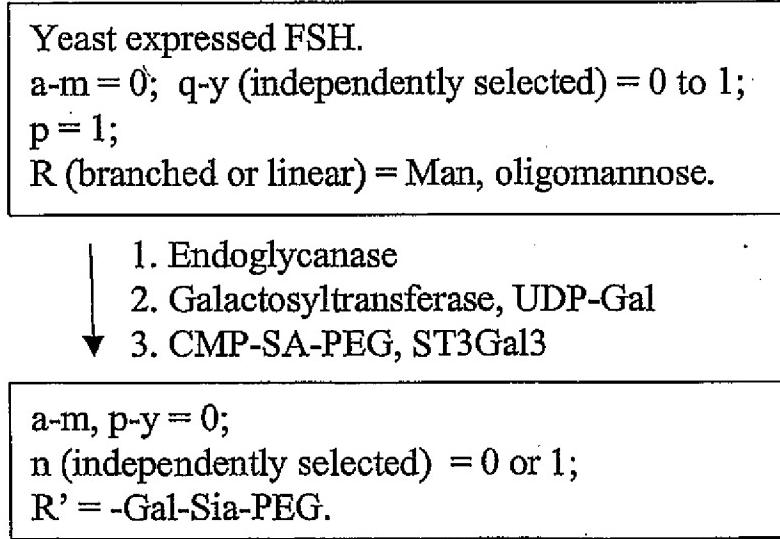
a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

## FIG. 32G

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## FIG. 32H



## FIG. 32I

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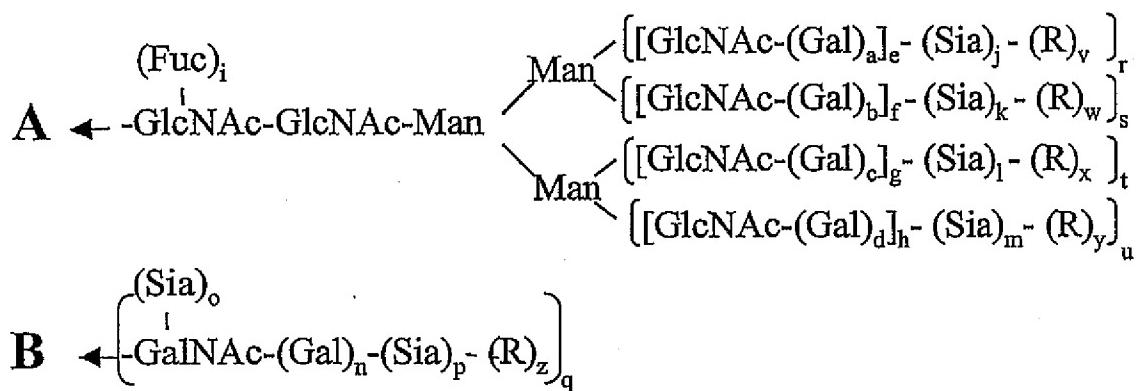
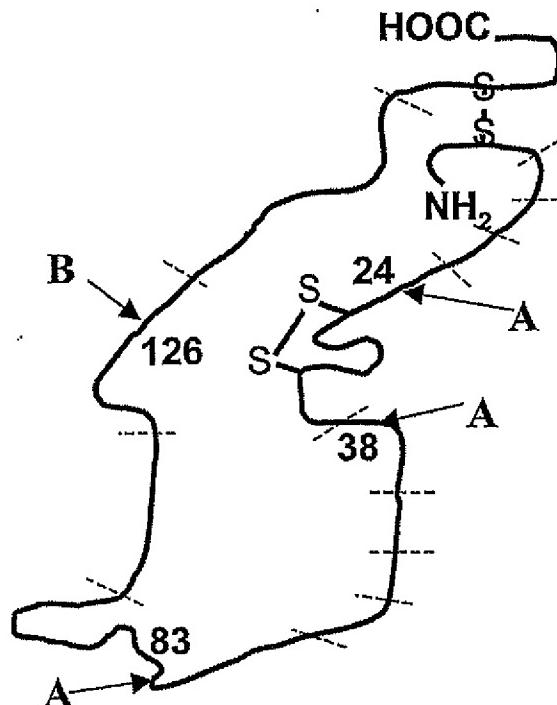
CHO, BHK, 293 cells, Vero expressed FSH.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP, ST3Gal3
  2. ST3Gal1, desialylated chorionic  
gonadotrophin (CG) produced in CHO.
  3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-CG.

FIG. 32J

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

v-z = 0;

R = modifying group, mannose, oligo-mannose.

FIG. 33A

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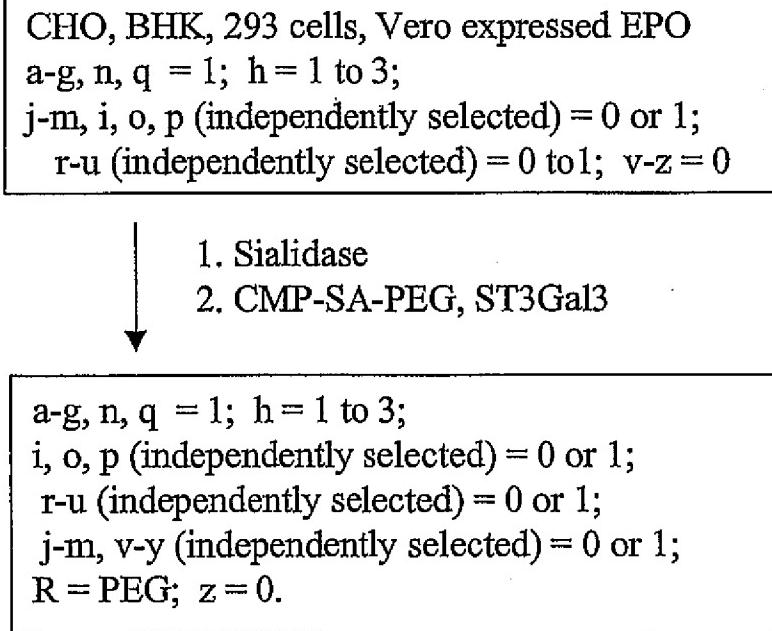


FIG. 33B

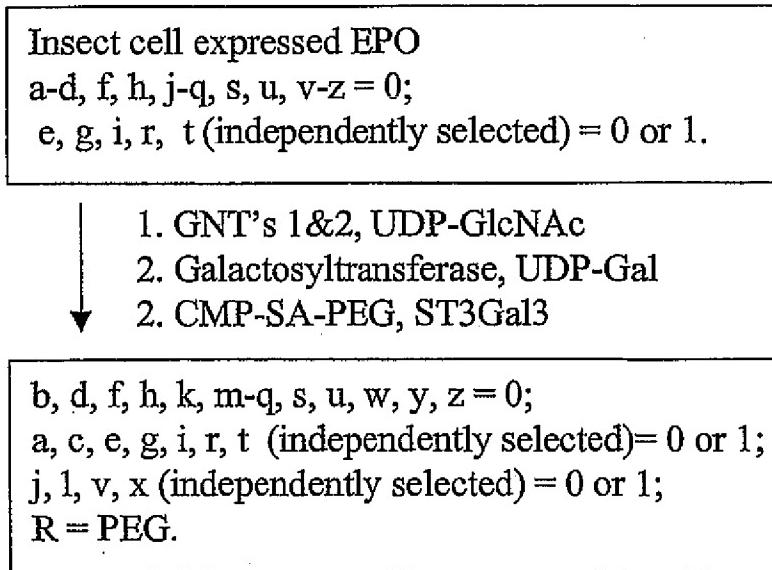


FIG. 33C

110/345

CHO, BHK, 293 cells, Vero expressed EPO  
a-q, r-u (independently selected) = 0 or 1;  
v-z = 0.

- ↓  
1. sialidase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA, ST3Gal3  
4. CMP-SA-PEG, ST3Gal1

a-h, n, q = 1;  
i-m, o, r-u (independently selected) = 0 or 1;  
v-y = 0; p, z = 0 or 1; R = PEG.

## FIG. 33D

CHO, BHK, 293 cells, Vero expressed EPO  
a-g, n, q = 1; h = 1 to 3;  
j-m, i, o, p (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
v-z = 0

- ↓  
1. CMP-SA-PEG, ST3Gal3

a-g, n, q = 1; h = 1 to 3;  
i, o, p (independently selected) = 0 or 1;  
r-u (independently selected) = 0 to 1;  
j-m, v-y (independently selected) = 0 or 1;  
R = PEG; z = 0.

## FIG. 33E

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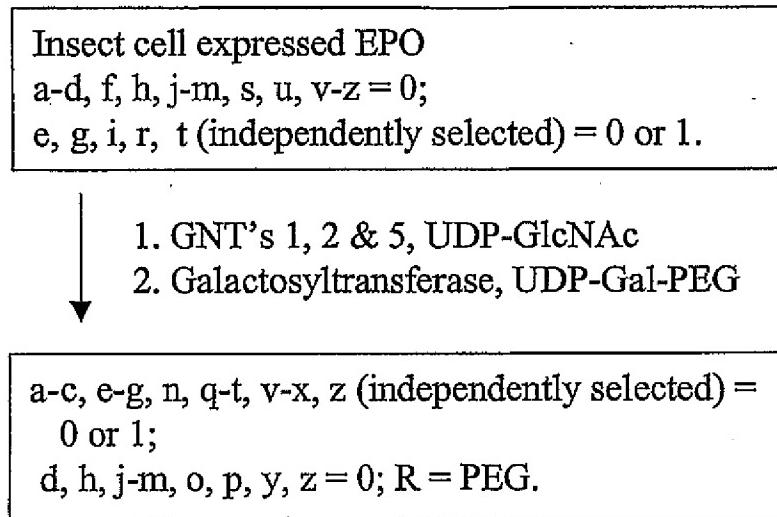


FIG. 33F

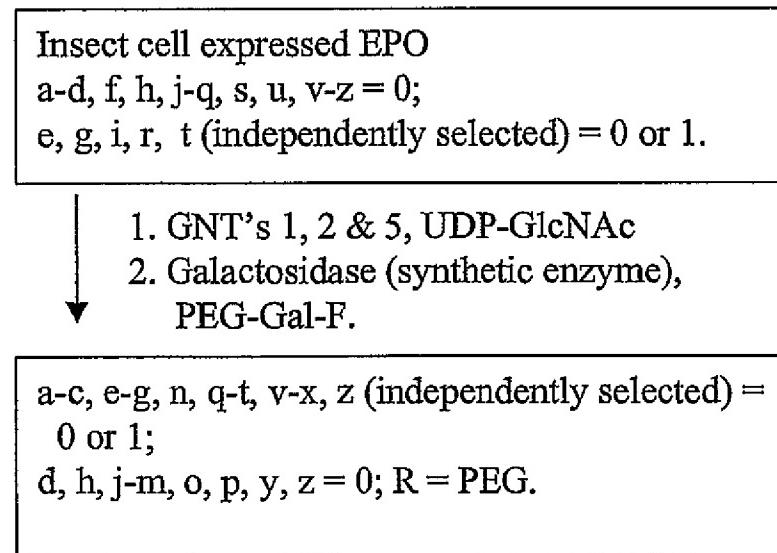


FIG. 33G

112/345

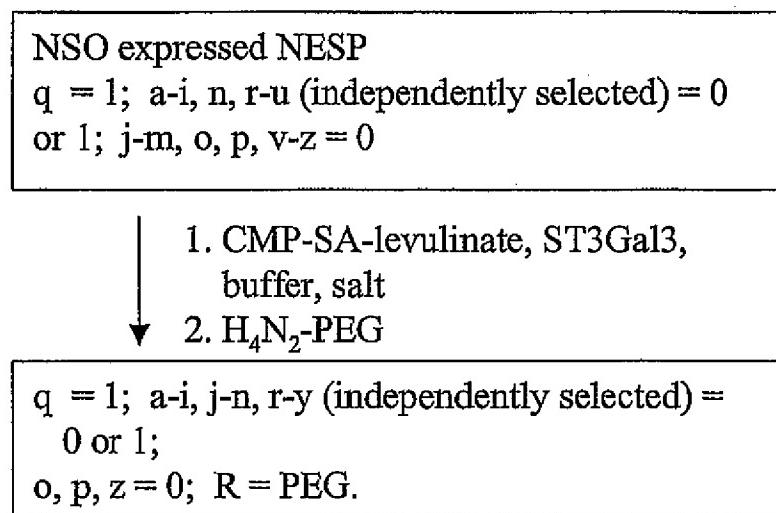


FIG. 33H

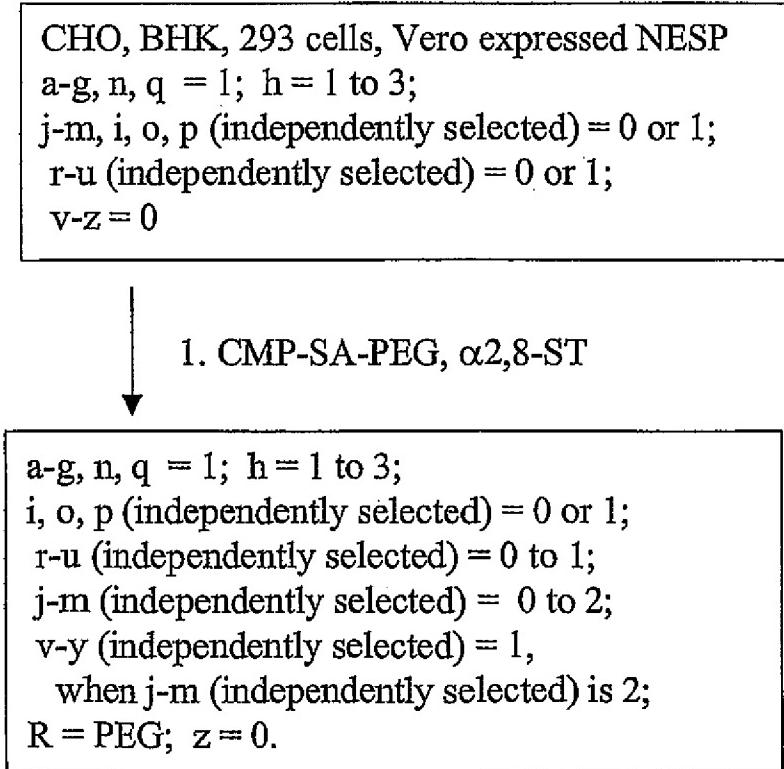


FIG. 33I

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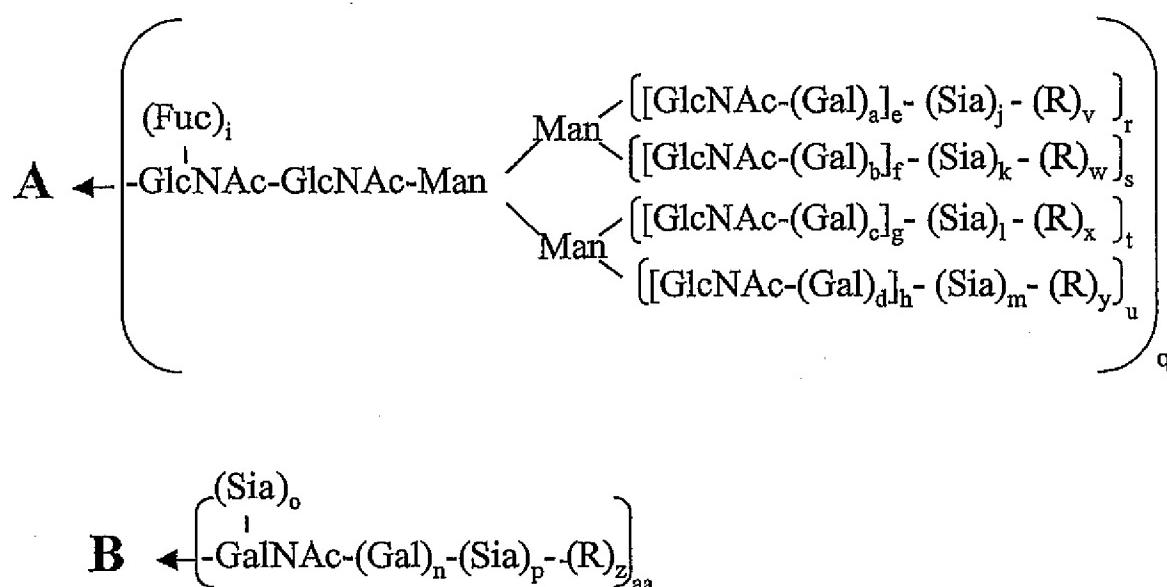
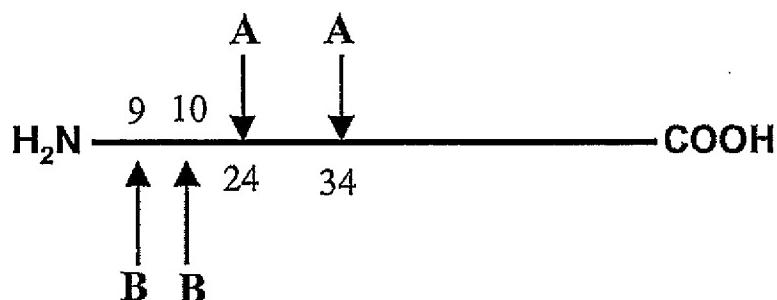
CHO, BHK, 293 cells, Vero expressed NESP  
a-g, n, q = 1; h = 1 to 3;  
j-m, i, o, p (independently selected) = 0 or 1;  
r-u (independently selected) = 0 to 1; v-z = 0

↓  
1. CMP-SA, poly- $\alpha$ 2,8-ST

a-g, n, q = 1; h = 1 to 3;  
i, j-m, o, p, r-u, (independently selected) = 0 or 1;  
v-z (independently selected) = 0-40; R = Sia.

FIG. 33J

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a-d, i, n-u, aa (independently selected) = 0 or 1.  
e-h (independently selected) = 0 to 6.  
j-m (independently selected) = 0 to 100.  
v-y = 0; R = polymer, glycoconjugate.

FIG. 34A

115/345

CHO, BHK, 293 cells, Vero expressed GM-CSF.  
 a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
 n, e-h = 1; v-z = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG (16 mol eq),  
 ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1;  
 o, p, z = 0; n, e-h = 1;  
 v-y (independently selected) = 1,  
 when j-m (independently selected) is 1;  
 R = PEG.

## FIG. 34B

CHO, BHK, 293 cells, Vero expressed GM-CSF.  
 a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
 n, e-h = 1; v-z = 0.

- ↓  
 1. Sialidase  
 2. CMP-SA-PEG (1.2 mol eq),  
 ST3Gal3  
 ↓ 3. CMP-SA (16 mol eq), ST3Gal3 &  
 ST3Gal1

a-d, i-m, p-u, aa (independently selected) = 0 or 1;  
 o, z = 0; n, e-h = 1;  
 v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 34C

116/345

NSO expressed GM-CSF.

a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0;  
Sia (independently selected) = Sia or Gal.

- ↓
1. Sialidase and  $\alpha$ -galactosidase
  2. CMP-SA, ST3Gal3
  2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1;  
n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

## FIG. 34D

CHO, BHK, 293 cells, Vero expressed GM-CSF.

a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-y, aa (independently selected) = 0 or 1;  
o, p, z = 0; n, e-h = 1; R = PEG.

## FIG. 34E

117/345

CHO, BHK, 293 cells, Vero expressed GM-CSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.



1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1;  
z = 0; n, e-h = 1; R = PEG.

## FIG. 34F

CHO, BHK, 293 cells, Vero expressed GMCSF.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.

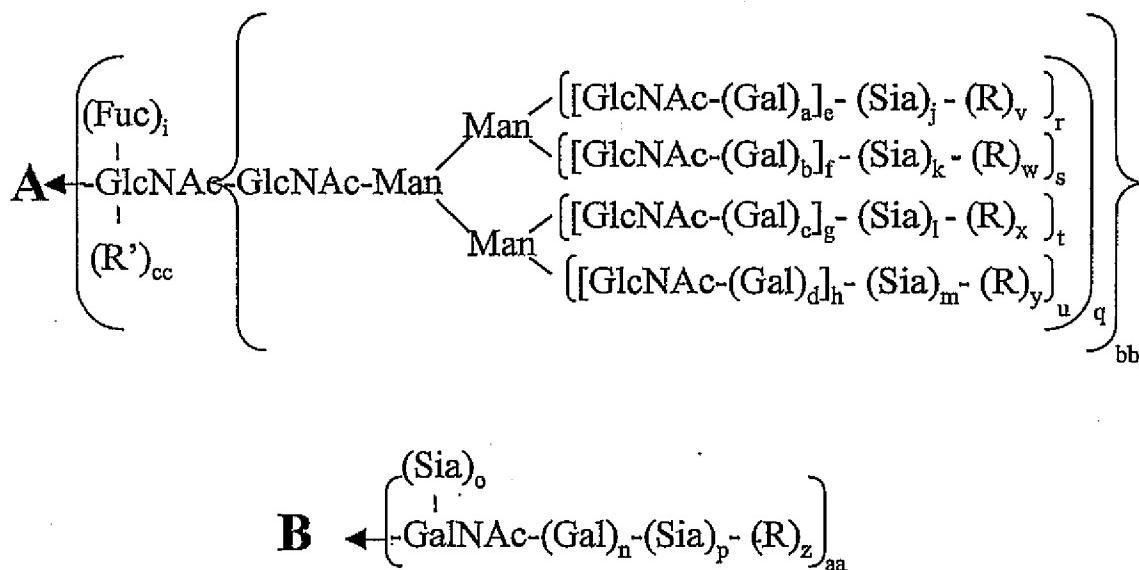
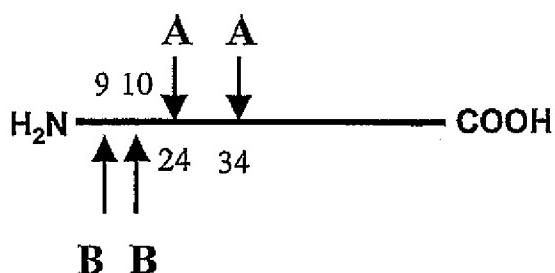


1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; j-m (independently selected) = 0-20;  
v-z (independently selected) = 0.

## FIG. 34G

118/345



a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = modifying group, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 34H

119/345

Insect cell expressed GM-CSF.  
 a-d, f, h, j-m, o, p, s, u, v-z = 0;  
 e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.

- ↓  
 1. GNT's 1,2,4,5, UDP-GlcNAc  
 2. Galactosyltransferase, UDP-Gal-PEG

a-i, n, q-u (independently selected) = 0 or 1;  
 j-m = 0; v-y (independently selected) = 1,  
 when e-h (independently selected) is 1;  
 R = PEG.

FIG. 34I

Yeast expressed GM-CSF.  
 a-p, z, cc = 0;  
 q-y, aa (independently selected) = 0 to 1;  
 bb = 1; R (branched or linear) = Man, oligomannose;  
 GalNAc = Man.

- ↓  
 1. Endoglycanase  
 2. mannosidase (if aa = 1).  
 3. Galactosyltransferase, UDP-Gal-PEG

a-p, r-z, aa, bb = 0;  
 q, cc (independently selected) = 0 or 1;  
 R' = -Gal-PEG.

FIG. 34J

120/345

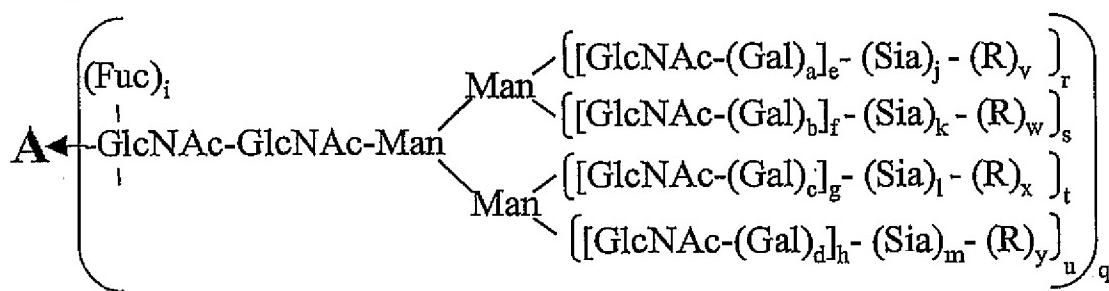
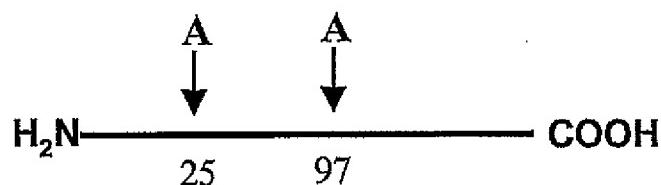
CHO, BHK, 293 cells, Vero expressed GM-CSF.  
a--m, o-u, aa, bb (independently selected) = 0 or 1;  
n, v-z, cc = 0.

- ↓
1. sialidase
  2. CMP-SA, ST3Gal3
  2. CMP-SA-linker-SA-CMP, ST3Gal1
  3. ST3Gal3, transferrin

a--m, p-u, z, aa (independently selected) = 0 or 1;  
o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 34K

121/345



a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 35A

122/345

CHO, BHK, 293 cells, Vero expressed IF-gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 35B

CHO, BHK, 293 cells, Vero expressed IF-gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 35C

123/345

NSO expressed Interferon gamma.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y = 0;  
 Sia (independently selected) = Sia or Gal.

- ↓
1. Sialidase and  $\alpha$ -galactosidase
  2.  $\alpha$ -Galactosyltransferase, UDP-Gal
  3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y (independently selected) = 1,  
 when j-m (independently selected) is 1;  
 R = PEG.

## FIG. 35D

CHO, BHK, 293 cells, Vero expressed  
 Interferon gamma.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
 ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y (independently selected) = 0 or 1;  
 R = PEG.

## FIG. 35E

124/345

CHO, BHK, 293 cells, Vero expressed

Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.

- ↓
1. CMP-SA-levulinate, ST3Gal3,
  2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y (independently selected) = 0 or 1;

R = PEG.

## FIG. 35F

CHO, BHK, 293 cells, Vero expressed

Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.

- ↓
1. CMP-SA, α2,8-ST

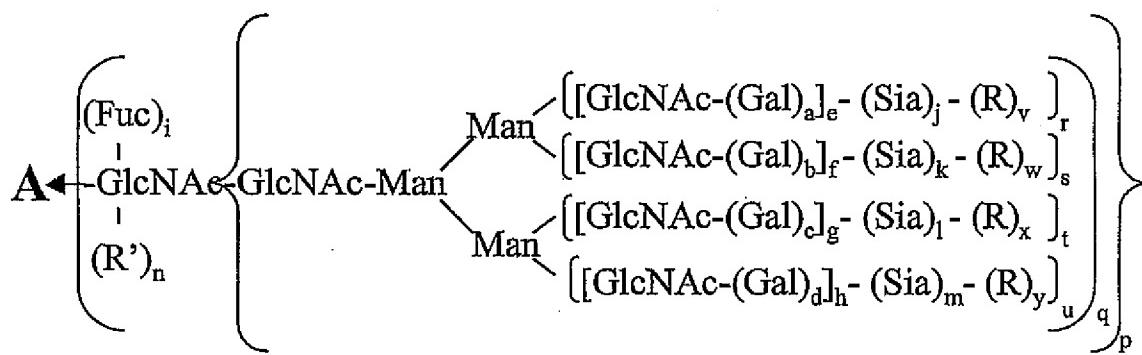
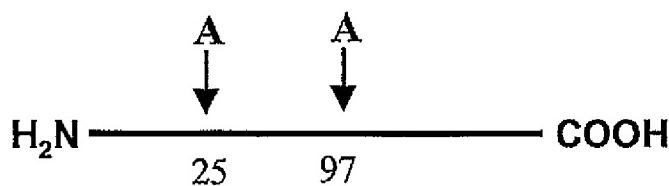
a-d, i, q-u (independently selected) = 0 or 1;

e-h = 1; j-m (independently selected) = 0-20;

v-y (independently selected) = 0.

## FIG. 35G

125/345



a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

$$\mathbf{v} - \mathbf{v} = \mathbf{0};$$

R = modifying group, mannose, oligo-mannose:

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 35H

126/345

Insect or fungi cell expressed IF-gamma.

a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

## FIG. 35I

Yeast expressed IF-gamma.

a-m = 0; q-y (independently selected) = 0 to 1; p = 1;  
R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

## FIG. 35J

127/345

CHO, BHK, 293 cells, Vero expressed IF-gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

↓  
1. CMP-SA-linker-Gal-UDP, ST3Gal3  
2. Galactosyltransferase, transferrin treated  
with endoglycanase.

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

## FIG. 35K

CHO, BHK, 293 cells, Vero expressed  
Interferon gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h, p = 1; n, v-y = 0.

↓  
1. CMP-SA-PEG,  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h, p = 1;  
n, v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 35L

128/345

Insect or fungi cell expressed IF-gamma.  
a-d, f, h, j-n, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

↓  
1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;  
e, g, i, r, t, q (independently selected) = 0 or 1;  
p = 1; v, x (independently selected) = 1,  
when e, g (independently selected) is 1;  
R = PEG.

## FIG. 35M

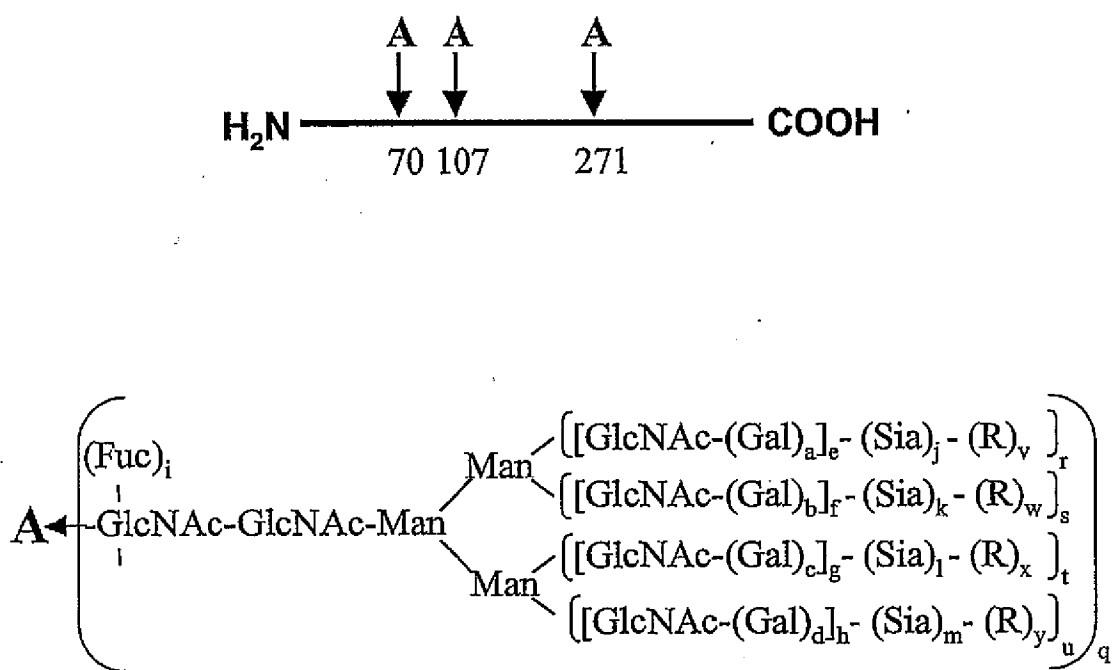
CHO, BHK, 293 cells, Vero expressed  
Interferon gamma.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

↓  
1. CMP-SA-PEG,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-2;  
v-y (independently selected) = 1,  
when j-m (independently selected) = 2;  
R = PEG.

## FIG. 35N

129/345



a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 36A

130/345

CHO, BHK, 293 cells, Vero or transgenic animal  
expressed  $\alpha_1$  antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 36B

CHO, BHK, 293 cells, Vero or transgenic  
animal expressed  $\alpha_1$  antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 36C

131/345

NSO expressed  $\alpha_1$ -antitrypsin.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y = 0;  
 Sia (independently selected) = Sia or Gal.

- ↓
1. Sialidase and  $\alpha$ -galactosidase
  2.  $\alpha$ -Galactosyltransferase, UDP-Gal
  3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1;  
 v-y (independently selected) = 1,  
 when j-m (independently selected) is 1;  
 R = PEG.

## FIG. 36D

CHO, BHK, 293 cells, Vero or transgenic animal  
 expressed alpha-1 antitrypsin.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
 ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y (independently selected) = 0 or 1;  
 R = PEG.

## FIG. 36E

132/345

CHO, BHK, 293 cells, Vero or transgenic animal  
expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt  
2.  $\text{H}_4\text{N}_2$ -PEG

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 36F

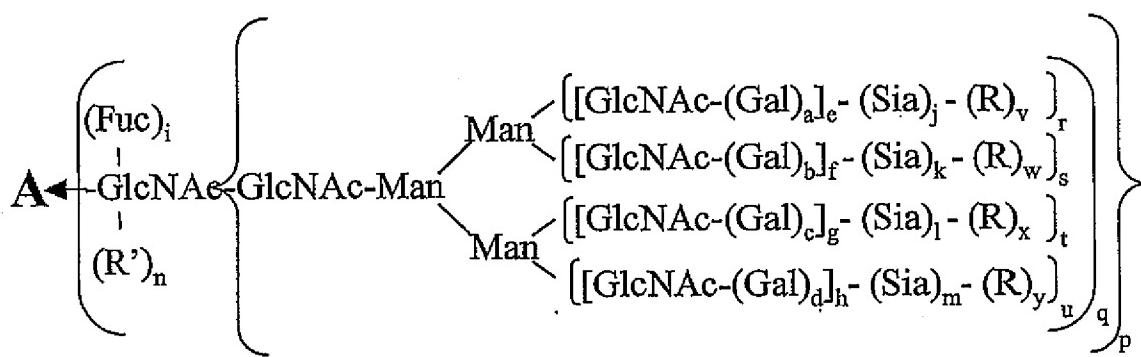
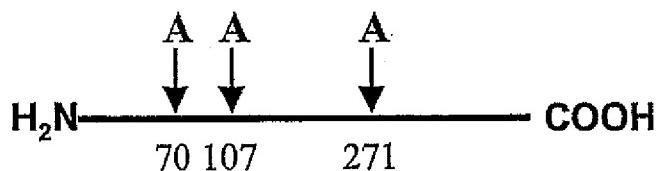
CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1;  
j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

## FIG. 36G

133/345



a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

$$v - v = 0;$$

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 36H

134/345

Insect or fungi cell expressed  $\alpha_1$ -antitrypsin.  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-m = 0;  
v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

## FIG. 36I

Yeast expressed  $\alpha_1$ -antitrypsin.  
a-m = 0; q-y (independently selected) = 0 to 1;  
p = 1; R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

## FIG. 36J

135/345

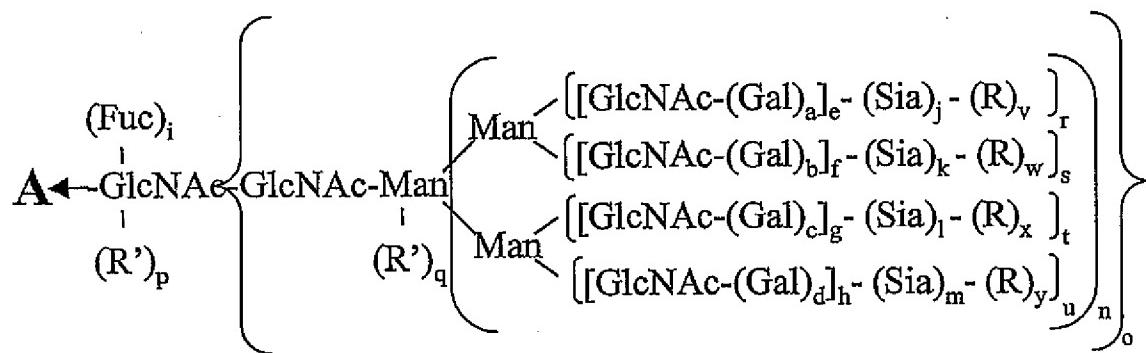
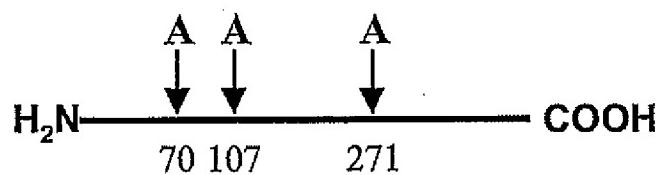
CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-Gal-UDP,  
ST3Gal3
  2. Galactosyltransferase, transferrin treated  
with endoglycanase

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 36K

136/345



a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer;

R', R'' (independently selected) = sugar, glycoconjugate.

**FIG. 36L**

137/345

Yeast expressed alpha-1 antitrypsin.  
a-h, i-m, p, q = 0;  
R (independently selected) = mannose, oligomannose,  
polymannose;  
r-u, v-y (independently selected) = 0 or 1; n, o = 1.

- ↓  
1. endoglycanase  
2. Galactosyltransferase, UDP-Gal-PEG

a-h, i-o, q, r-u, v-y = 0; p = 1.  
R'' = Gal-PEG.

## FIG. 36M

Plant expressed alpha-1 antitrypsin.  
a-d, f, h, j- m, s, u , v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1;  
n=1; R' = xylose

- ↓  
1. hexosaminidase,  
2. alpha mannosidase and xylosidase  
3. GlcNAc transferase, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u , v-y = 0;  
e, g, i, r, t (independently selected) = 0;  
q = 1; R' = GlcNAc-PEG.

## FIG. 36N

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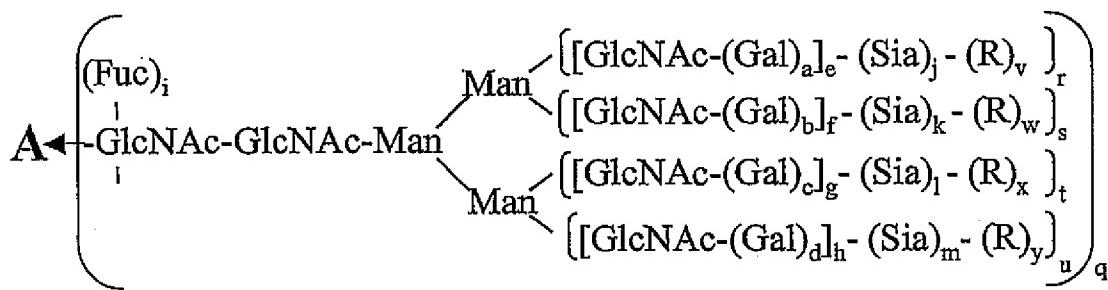
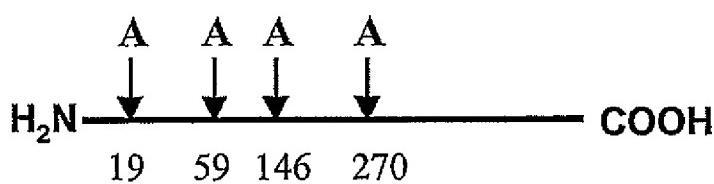
CHO, BHK, 293 cells, Vero, transgenic animal  
expressed  $\alpha_1$  antitrypsin.  
a-h, i-o, r-u (independently selected) = 0 or 1;  
p, q, v-y = 0.

↓  
1. CMP-SA-PEG,  
ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1;  
p, q = 0; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 36O

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 37A

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CHO, BHK, 293 cells, Vero expressed Cerezyme  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 37B

CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-M-6-P (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = mannose-6-phosphate

## FIG. 37C

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NSO expressed Cerezyme.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.

1. Sialidase and  $\alpha$ -galactosidase
2.  $\alpha$ -Galactosyltransferase, UDP-Gal
3. CMP-SA-M-6-P, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = mannose-6 phosphate

## FIG. 37D

CHO, BHK, 293 cells, Vero expressed Cerezyme.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y (independently selected) = 0 or 1;

R = Mannose-6-phosphate

## FIG. 37E

142/345

CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt.
  2. H<sub>4</sub>N<sub>2</sub>-spacer-M-6-P or clustered M-6-P

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = M-6-P or clustered M-6-P

## FIG. 37F

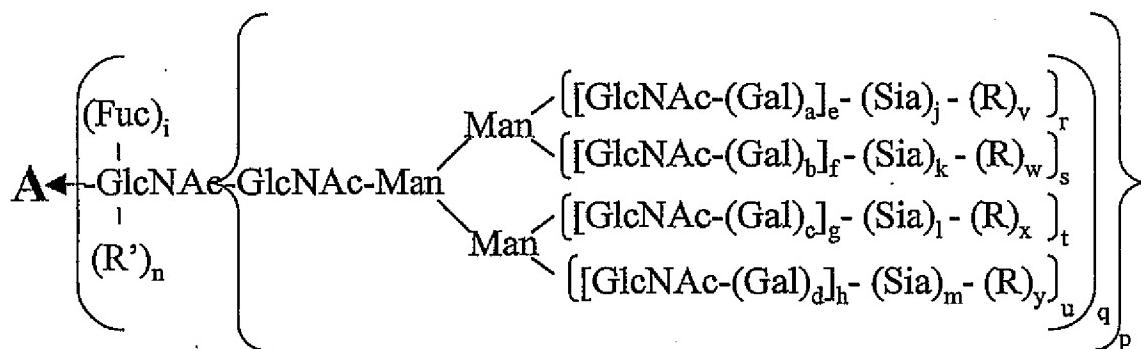
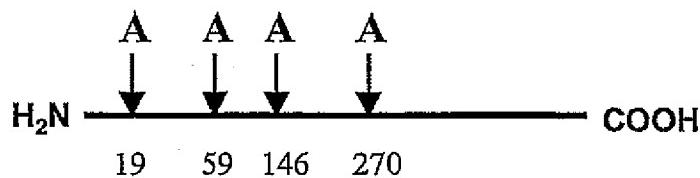
CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

## FIG. 37G

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

**FIG. 37H**

144/345

Insect cell expressed Cerezyme.  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0;  
v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

## FIG. 37I

Yeast expressed Cerezyme.  
a-m = 0; q-y (independently selected) = 0 to 1;  
p = 1; R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;  
R' = -Gal-Sia-PEG.

## FIG. 37J

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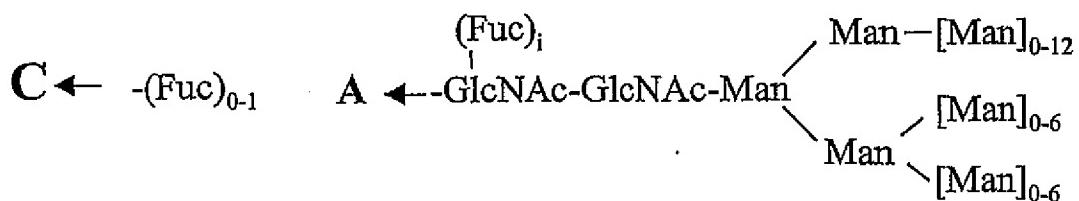
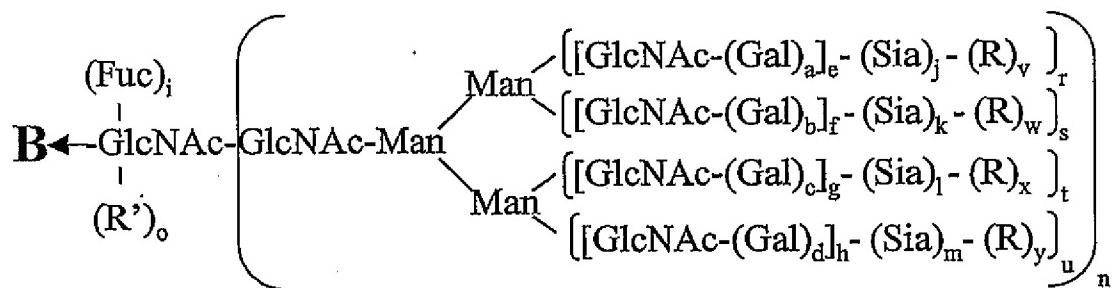
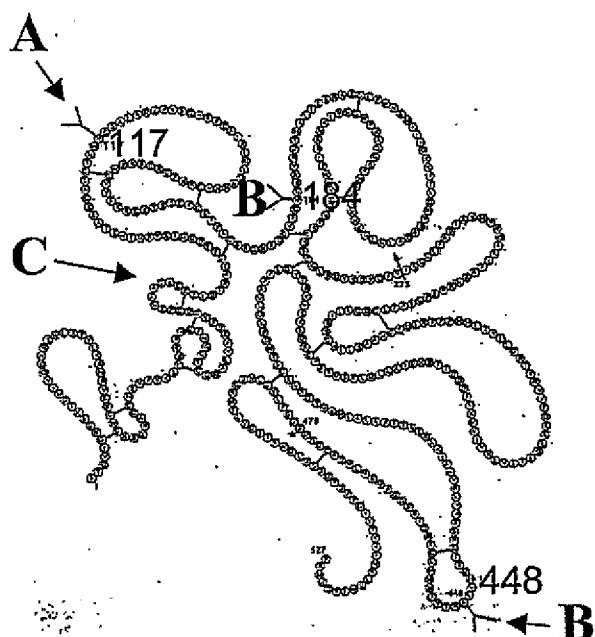
CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP,  
ST3Gal3
  2. ST3Gal3, desialylated transferrin.
  3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0; v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 37K

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer; R' = sugar, glycoconjugate.

FIG. 38A

147/345

CHO, BHK, 293 cells, Vero expressed tPA  
 a-g, n = 1; h = 1 to 3;  
 j-m, i, (independently selected) = 0 or 1;  
 r-u (independently selected) = 0 to 1; o, v-y = 0.

- 1. Mannosidase(s), sialidase
- 2. GNT1,2 (4 and/or 5) UDP-GlcNAc
- 3. Gal transferase, UDP-Gal
- 4. CMP-SA-PEG, ST3Gal3

A = B; a-g, n = 1; h = 1 to 3;  
 i, r-u (independently selected) = 0 or 1;  
 o = 0; j-m, v-y (independently selected) = 0 or 1;  
 R = PEG

## FIG. 38B

Insect or fungi cell expressed tPA  
 A = B; a-d, f, h, j-o, s, u, v-y = 0;  
 e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

A = B; b, d, f, h, k, m, o, s, u, w, y = 0;  
 a, c, e, g, i, r, t (independently selected) = 0 or 1;  
 n = 1; j, l, v, x (independently selected) = 0 or 1;  
 R = PEG.

## FIG. 38C

148/345

Yeast expressed tPA

$B = A; i = 0.$

- ↓
1. endoglycanase
  2. Galactosyltransferase,  
    UDP-Gal-PEG

$A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.$

## FIG. 38D

Insect or fungi cell expressed tPA

$A = B; a-d, f, h, j-o, s, u, v-y = 0;$   
 $e, g, i, n, r, t$  (independently selected) = 0 or 1.

- ↓
1. alpha and beta mannosidases
  2. Galactosyltransferase, UDP-Gal-PEG

$A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.$

## FIG. 38E

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Insect or fungi cell expressed tPA  
A = B; a-d, f, h, j-o, s, u, v-y = 0;  
e, g, i, n, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1&2, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

A = B; b, d, f, h, j-o, s, u, w, y = 0;  
a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1;  
n = 1; R = PEG.

## FIG. 38F

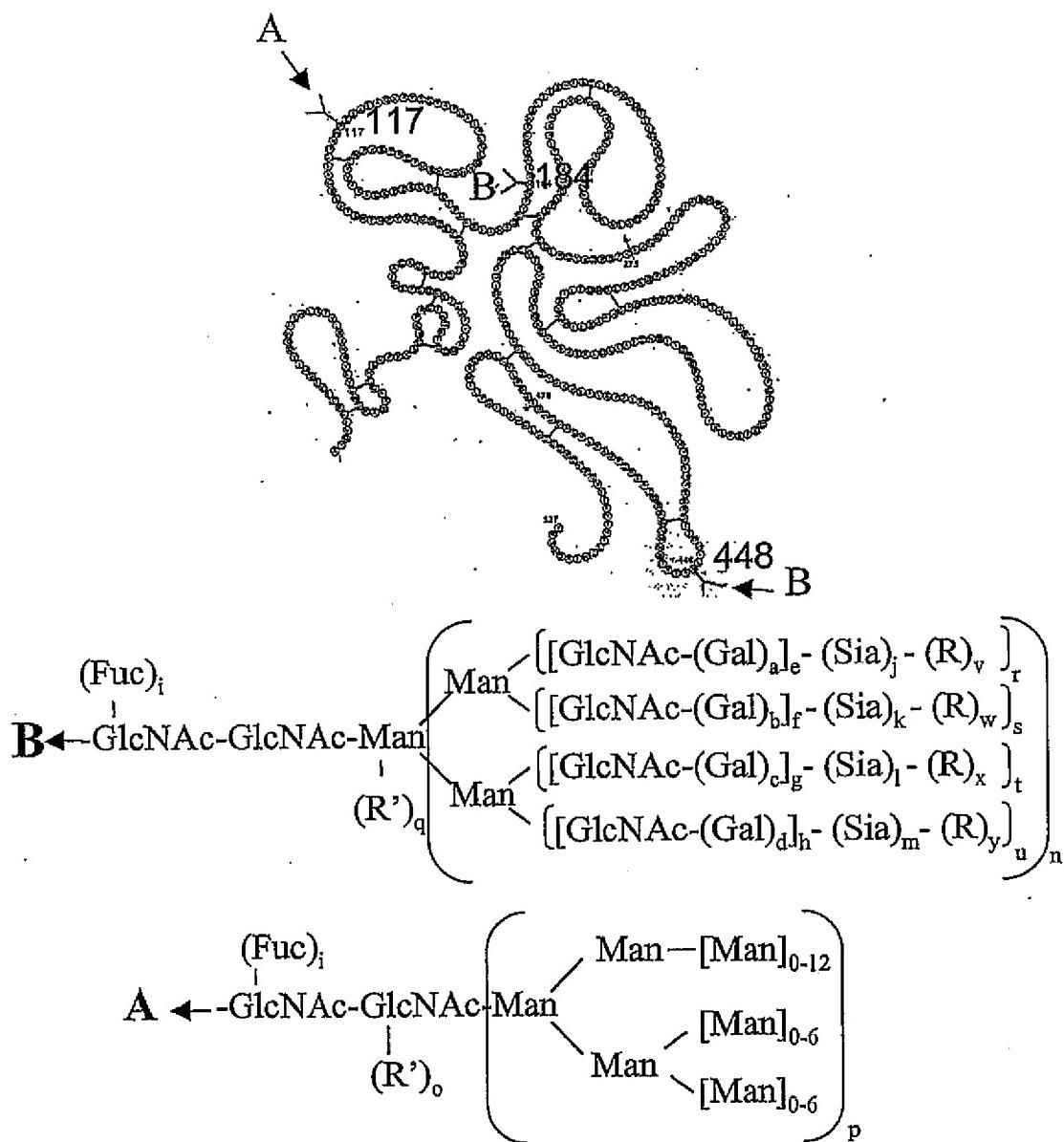
Insect or fungi cell expressed tPA  
A = B; a-d, f, h, j-o, s, u, v-y = 0;  
e, g, i, n, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1 & 2, UDP-GlcNAc  
2. Galactosidase (synthetic enzyme),  
PEG-Gal-F.

A = B; b, d, f, h, j-o, s, u, w, y = 0;  
a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1;  
n = 1; R = PEG.

## FIG. 38G

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a-d, i, n-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 4.  
 j-m (independently selected) = 0 to 20.  
 R = polymer; R' = sugar, glycoconjugate.

FIG. 38H

151/345

NSO expressed tPA

$A = B$ ;  $a-m, r-u$  (independently selected) = 0 or 1;  
 $n = 1$ ;  $o, p, q, v-y = 0$

- ↓
1. sialidase, alpha-galactosidase
  2. CMP-SA-levulinate, ST3Gal3,
  3.  $H_4N_2$ -PEG

$A = B$ ;  $a-m, r-y$  (independently selected) = 0 or 1;  
 $n = 1$ ;  $o, p, q = 0$ ;  
 $v-y$  (independently selected) = 1,  
when  $j-m$  (independently selected) is 1;  
 $R = PEG$ .

## FIG. 38I

CHO, BHK, 293 cells, Vero expressed tPA

$a-g, n, p = 1$ ;  $h = 1$  to 3;  
 $j-m, i$ , (independently selected) = 0 or 1;  
 $r-u$  (independently selected) = 0 to 1;  $q, o, v-y = 0$ .

- ↓
1. alpha and beta Mannosidases
  2. CMP-SA, ST3Gal3
  3. Galactosyltransferase, UDP-Gal-PEG

$a-g, n = 1$ ;  $h = 1$  to 3;  
 $i, r-u$  (independently selected) = 0 or 1;  $o = 1$ ;  
 $q, p, v-y = 0$ ;  $j-m$  (independently selected) = 0 or 1;  
 $R' = Gal-PEG$

## FIG. 38J

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Plant expressed tPA

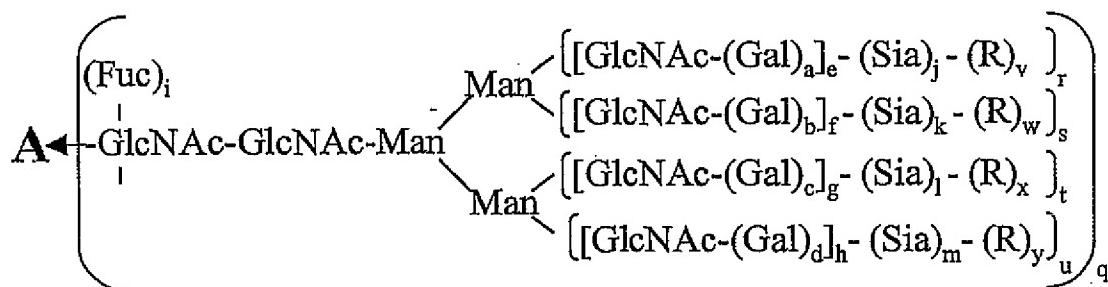
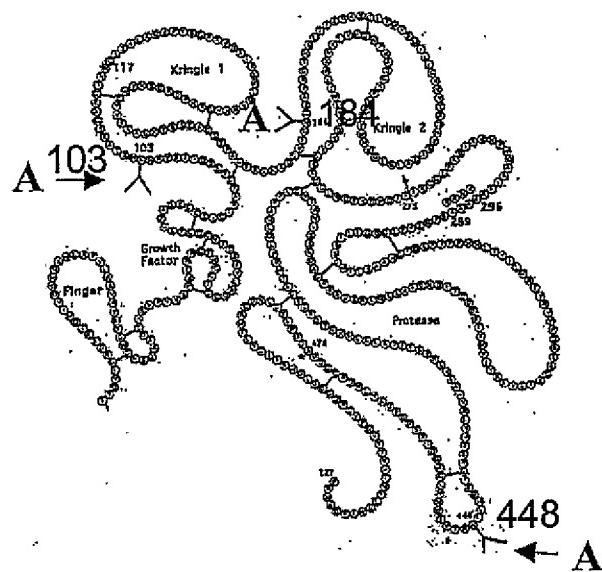
A = B; a-d, f, h, j- m, s, u , v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1;  
n =1; R' = xylose

- ↓
1. hexosaminidase,
  2. alpha mannosidase and  
xylosidase
  3. GlcNAc transferase, UDP-  
GlcNAc-PEG

A = B; a-d, f, h, j-n, s, u , v-y = 0;  
e, g, i, r, t (independently selected) = 0;  
q = 1; R' = GlcNAc-PEG.

FIG. 38K

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 38L

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CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

## FIG. 38M

CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓  
1. Sialidase  
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3  
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 38N

155/345

NSO expressed TNK tPA

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.

1. Sialidase and  $\alpha$ -galactosidase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;

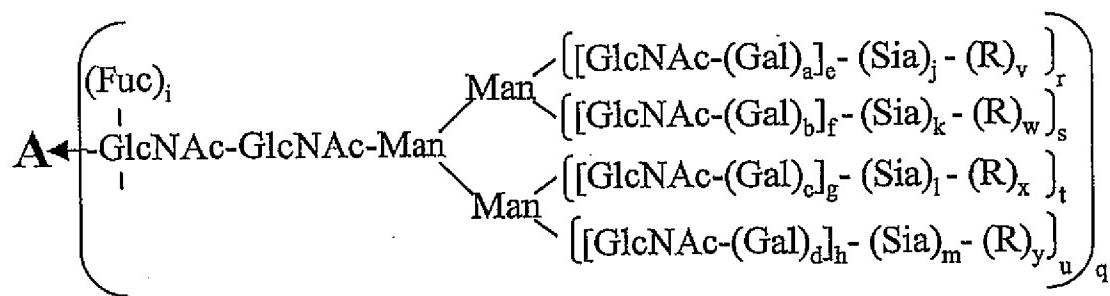
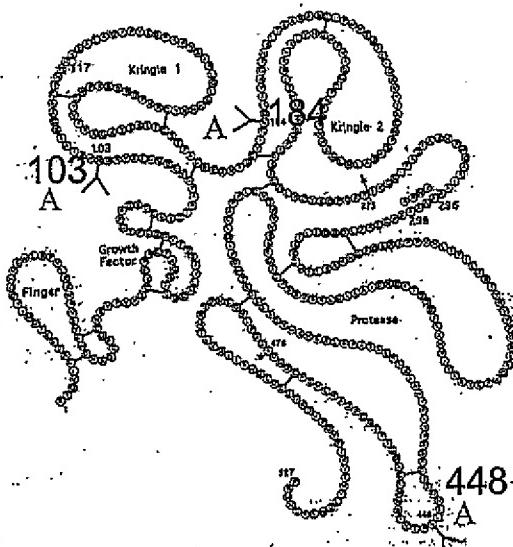
e-h = 1; v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = PEG.

FIG. 38O

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 38P

157/345

CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 38Q

CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt
  2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

## FIG. 38R

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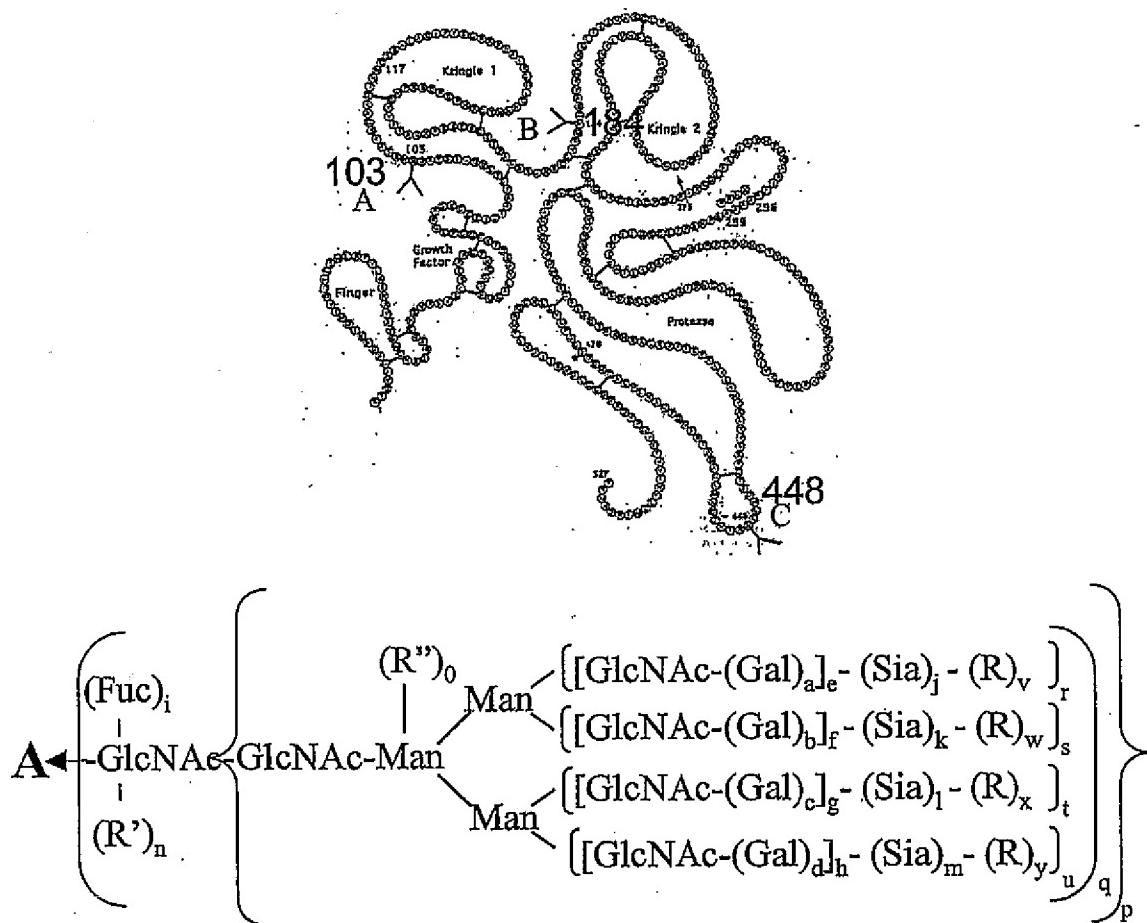
CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

↓  
1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 38S

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a-d, i, n-y (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

R'' = glycosyl residue.

FIG. 38T

160/345

Insect cell expressed TNK tPA  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓  
1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

## FIG. 38U

Yeast expressed TNK tPA  
a-m = 0; q-y (independently selected) = 0 to 1; p = 1;  
R (branched or linear) = Man, oligomannose.

- ↓  
1. Endoglycanase  
2. Galactosyltransferase, UDP-Gal-PEG

a-m, p-y = 0; n (independently selected) = 0 or 1;  
R' = -Gal-PEG.

## FIG. 38V

161/345

CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-Gal-UDP,  
ST3Gal3
  2. Galactosyltransferase, anti-TNF  
IG chimera produced in CHO.

a-m, r-u (independently selected) = 0 or 1; p, q = 1;  
n = 0; v-y (independently selected) = 0 or 1;  
R = linker-anti-TNF IG chimera protein.

FIG. 38W